

-37-

**What Is Claimed Is:**

Cancelled  
see claims filed  
66/2797 - paper # 21

1           1.     A method for preparing correctly folded and disulfide bond-  
2 linked interferon- $\alpha$  by expression in *E. coli*, comprising the steps of:

- 3           (a)    expressing interferon- $\alpha$  in *E. coli* comprising a vector  
4                    in which the signal sequence of the gene for the heat  
5                    stable enterotoxin II (STII) from *E. coli* is operably  
6                    linked to a sequence which codes for mature human  
7                    interferon- $\alpha$ ; and  
8           (b)    isolating the expressed interferon- $\alpha$ .

1           2.     The method of claim 1, wherein said vector further comprises  
2 an *E. coli* alkaline phosphatase (*phoA*) promoter.

1           3.     The method of claim 1 wherein said vector further comprises  
2 the sequence for the ribosome binding site of the STII gene.

1           4.     The method of claim 1 wherein said isolating step comprises the  
2 steps of:

- 3           (a)    performing chromatography on silica gel;  
4           (b)    performing hydrophobic interaction chromatography;  
5           (c)    performing exchange chromatography; and  
6           (d)    performing exchange chromatography.

1           5.     The method of claim 4, wherein said hydrophobic interaction  
2 chromatography employs phenyl sepharose<sup>™</sup>.

1           6.     The method of claim 4, wherein said cation exchange  
2 chromatography employs a sulfonated ion exchange resin.

-38-

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B 1 7. The method of claim 4, wherein said anion exchange  
2 chromatography employs DBAE-sepharose<sup>TM</sup>.

1 8. The method of claim 1, wherein said interferon- $\alpha$  is  
2 interferon- $\alpha$ 2.

B 1 9. The method of claim 8, wherein said interferon- $\alpha$ 2 <sup>comprising</sup> consists  
B 2 essentially of the sequence:

3 Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr  
4 Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser  
5 Cys Leu Lys Asp Arg Arg Asp Phe Gly Phe Pro Gln Glu Glu  
6 Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu  
7 His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys  
8 Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe  
9 Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys  
10 Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys  
11 Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile  
12 Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp  
13 Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser  
14 Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu (SEQ ID NO:5).

1 10. A method of purifying interferon- $\alpha$ , comprising the steps of:  
2 (a) performing chromatography of crude interferon- $\alpha$  on  
3 silica gel;  
4 (b) performing hydrophobic interaction chromatography on  
5 the interferon- $\alpha$  obtained in step (a);  
6 (c) performing exchange chromatography on the interferon-  
7  $\alpha$  obtained in step (b); and  
8 (d) performing exchange chromatography on the interferon-  
9  $\alpha$  obtained in step (c);  
10 whereby purified interferon is obtained.

- 1 11. The method of claim 10, wherein said hydrophobic interaction  
2 chromatography employs phenyl sepharose.
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-39-

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1 12. The method of claim 10, wherein said cation exchange  
2 chromatography employs sulphopropyl ion exchanger.

1 13. The method of claim 10, wherein said anion exchange  
2 chromatography employs DEAE-sepharose.

1 14. The method of claim 10, wherein said crude interferon- $\alpha$  was  
2 expressed in bacteria.

1 15. The method of claim 10, wherein said interferon- $\alpha$  is  
2 interferon- $\alpha 2$ .

1 16. The method of claim 15, wherein said interferon- $\alpha 2$  consists  
2 essentially of the sequence:

3 Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr  
4 Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser  
5 Cys Leu Lys Asp Arg Arg Asp Phe Gly Phe Pro Gln Glu Glu  
6 Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu  
7 His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys  
8 Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe  
9 Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys  
10 Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys  
11 Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile  
12 Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp  
13 Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser  
14 Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu (SEQ ID NO:7).

1 17. A vector for expressing interferon- $\alpha$  in *E. coli*, comprising a  
2 DNA molecule coding for the signal sequence of the STII gene operably  
3 linked to a DNA molecule which codes for mature human interferon- $\alpha$ .

*Sub  
B4*

- 1 18. The vector of claim 17, wherein said vector further comprises  
2 a phoA promoter.
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-40-

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1 19. The vector of claim 17, wherein said vector further comprises  
2 a ribosome binding site of the STII gene.

1 20. The vector of claim 17, wherein said interferon- $\alpha$  is  
2 interferon- $\alpha$ 2.

1 21. The vector of claim 17, wherein said DNA molecule coding for  
2 interferon- $\alpha$  consists essentially of the sequence:

*Sub B5*

3 TGT GAT CTG CCT CAA ACC CAC AGC CTG GGT AGC AGG AGG ACC  
4 TTG ATG CTC CTG GCA CAG ATG AGG AGA ATC TCT CTT TTC TCC  
5 TGC TTG AAG GAC AGA CGT GAC TTT GGA TTT CCC CAG GAG GAG  
6 TTT GGC AAC CAG TTC CAA AAG GCT GAA ACC ATC CCT GTC CTC  
7 CAT GAG ATG ATC CAG CAG ATC TTC AAT CTC TTC AGC ACA AAG  
8 GAC TCA TCT GCT GCT TGG GAT GAG ACC CTC CTA GAC AAA TTC  
9 TAC ACT GAA CTC TAC CAG CAG CTG AAT GAC CTG GAA GCC TGT  
10 GTG ATA CAG GGG GTG GGG GTG ACA GAG ACT CCC CTG ATG AAG  
11 GAG GAC TCC ATT CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC  
12 ACT CTC TAT CTG AAA GAG AAG AAA TAC AGC CCT TGT GCC TGG  
13 GAG GTT GTC AGA GCA GAA ATC ATG AGA TCT TTT TCT TTG TCA  
14 ACA AAC TTG CAA GAA AGT TTA AGA AGT AAG GAA (SEQ ID NO:6)

15 or a sequence which is more than about 70% homologous with this sequence  
16 and said sequence which is said homologous codes for interferon- $\alpha$ .

1 22. The vector of claim 17, wherein said DNA molecule coding for  
2 interferon- $\alpha$  consists essentially of the sequence:

3 TGT GAT CTG CCT CAA ACC CAC AGC CTG GGT AGC AGG AGG ACC  
4 TTG ATG CTC CTG GCA CAG ATG AGG AGA ATC TCT CTT TTC TCC  
5 TGC TTG AAG GAC AGA CGT GAC TTT GGA TTT CCC CAG GAG GAG  
6 TTT GGC AAC CAG TTC CAA AAG GCT GAA ACC ATC CCT GTC CTC  
7 CAT GAG ATG ATC CAG CAG ATC TTC AAT CTC TTC AGC ACA AAG

-41-

11 GAG GAC TCC ATT CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC-  
12 ACT CTC TAT CTG AAA GAG AAG AAA TAC AGC CCT TGT GCC TGG  
13 GAG GTT GTC AGA GCA GAA ATC ATG AGA TCT TTT TCT TTG TCA  
14 ACA AAC TTG CAA GAA AGT TTA AGA AGT AAG GAA (SEQ ID NO:6)

15 or a sequence which is more than about 70% homologous with this sequence  
16 and said sequence which is said homologous codes for interferon- $\alpha$ .

1 23. A method for preparing correctly folded and disulfide bond-  
2 linked interferon- $\alpha$  by expression in *E. coli*, comprising the steps of:

- 3 (a) expressing interferon- $\alpha$  in *E. coli* comprising the vector  
4 of claim 30; and  
5 (b) isolating the expressed interferon- $\alpha$ .

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C3